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Long-term effects of neonatal pain and sucrose treatment

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ABSTRACT

Purpose: In neonatal intensive care units, applying sucrose solution for analgesia is now a routine treatment for mild procedural pain. Studies of animal and human infants provide clear evidence of benefits in the short term, but few studies have investigated the long term benefits. Thus, we determined whether sucrose could ameliorate painful stimulation during infancy in Sprague–Dawley rats and also explored the long-term effects of repeated sucrose administration during infancy. Female and male rats were included to investigate sex-related differences.
Methods: Rat pups were stimulated either with painful or tactile stimuli for the first 14 days of their lives. Pups were pretreated either with sucrose or not treated before stimulation. Behavioral tests were conducted during adolescence and adulthood. Hotplate, rotarod, open field, elevated plus maze, and radial arm water maze tests were employed to assess the behavioral consequences of early life manipulations and treatments.
Results: Painful stimulation during infancy increased the sensitivity to pain later in life, and sucrose did not remedy this effect. Motility, coordination, anxiety, and cognition tests in adulthood obtained mixed results. Pain during infancy appeared to increase anxiety during adulthood. Learning and memory in adulthood were affected by pain during infancy, and sucrose had a negative effect even in the absence of pain. No sex-related differences were observed in any of the behavioral tests by employing this model of neonatal pain.
Conclusion: Painful stimulation during infancy resulted in deficiencies in some behavioral tests later in life. Sucrose pretreatment did not mitigate these shortcomings and it actually resulted in negative outcomes.

1. Introduction

The management of neonatal pain is a critical health issue that is increasingly recognized by clinicians and researchers (Hall and Anand, 2014; Carter and Brunkhorst, 2017; Shah and Siu, 2019; Carbajal, 2020; Mencía et al., 2022). However, the use of classical pain medications has some adverse effects in both the short and long term, thereby making their use controversial (Bastaki et al., 2018; Shah and Siu, 201; Kinoshita et al., 2021). Therefore, identifying appropriate pain management methods for this vulnerable population is both ethically and clinically important. The effective non-pharmacological management of neonatal pain could address the aims of treating pain and preventing long-term detrimental effects (Mangat et al., 2018).

Sweet-tasting solutions have shown promise in the management of mild to moderately painful procedures in both human and non-human mature and premature infants (Gao et al., 2016; De Bernardo et al., 2019; Nuseir et al., 2022; Yamada et al., 2023). The mechanism associated with sweet-induced analgesia has not been fully elucidated but some studies suggest the involvement of the opioid system (Kakeda et al., 2010; K. Nuseir et al., 2017; Yamamotová, 2019), as well as other neuronal and hormonal factors (Irusta et al., 2001; Kishi et al., 2006; Davies et al., 2019). However, the use of sucrose and other sweeteners is not without possible adverse effects (Campbell et al., 2014; Gao et al., 2016). Therefore, it is important to understand the long-term effects of sucrose pretreatment for painful stimulation during infancy.

Historically, female animals have generally not been included in research, thereby leading to a lack of knowledge regarding sex-related differences in many research areas (Mogil and Chanda, 2005; Beery,

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2018; Ah-King, 2022). Therefore, including both sexes in studies is essential for obtaining a more comprehensive understanding of any potential sex-related differences in the response to painful stimulation and the use of sucrose for pain management in neonates. Recently, it has become increasingly important to actually include females in preclinical studies because extrapolations of the results obtained in these studies are applicable to women (Clayton, 2018). The perceived difficulties of including females in preclinical studies have been shown to be incorrect (Beery, 2018).

Despite the widespread use of sweet-tasting solutions to alleviate minor procedural pain, human and animal studies have demonstrated poor long-term outcomes related to this treatment. For example, a study of preterm infants administered with glucose for minor invasive procedures during their stay at a neonatal intensive care unit showed that glucose did not alleviate the detrimental effects of pain on brain development and structures (Schneider et al., 2018). Furthermore, pretreatment of neonatal mice with sucrose did not mitigate the long-term effects of pain. Moreover, sucrose given in the absence of pain resulted in worse outcomes later in life (Ranger et al., 2019a,b).

In our previous studies, we focused on the use of sucrose to decrease the long-term effects of neonatal pain in rats. Sucrose pretreatment resulted in better tolerance of painful stimuli later in life in a rat model of nociceptive and inflammatory pain (Nuseir et al., 2015, 2017, 2019). However, the effects on learning and memory were mixed, where sucrose pretreatment improved short-term memory compared with painful stimulation without treatment (Nuseir et al., 2015). By contrast, long-term memory was improved by sucrose pretreatment compared with painful stimuli during infancy in rats (K. Q. Nuseir et al., 2017). Sucrose treatment without pain induction had no effect on any of the tests in rats later in life.

Thus, in the present study, we aimed to evaluate the effects of repeated neonatal pain on Sprague–Dawley rats, the effects of repeated sucrose treatment with and without pain, and possible sex-related differences on sensitivity to pain.

The results obtained in this study can potentially provide valuable insights into the effectiveness and safety of sucrose for neonatal pain management, as well as the importance of considering sex-related differences in neonatal pain research.

2. Materials and methods

2.1. Animals and treatments

Timed-pregnant Sprague–Dawley rats obtained from Jordan University of Science and Technology Animal Facility were housed individually in standard metal cages containing wood chip bedding under hygienic conditions and maintained at 24 ± 1 °C and a 12 h/12 h light/dark cycle starting at 7 a.m., with food and water available *ad libitum*. Litters born on the same day within 24 h of each other were standardized to 5–8 pups per cage. Female and male rat pups were randomly distributed between cages immediately after birth and remained with their mothers until weaning on postnatal day 28 (p = 28). After weaning, males and females were housed separately to avoid copulation, with three to four rats per cage. All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize suffering and stress, such as returning pups to their cages between manipulations and using a minimum number of rats per experiment. All experimental procedures were approved by the Animal Care and Use Committee of Jordan University of Science and Technology (Grant Number: 2022/53).

Animals were randomly distributed into four groups (n = 7–18), and all manipulations were performed four times daily at hourly intervals for 14 days. All manipulations were initiated on day one of birth (P0). This procedure was used previously in our laboratory based on a study by Anand et al. (1999), who noted that fewer than four pricks did not result in any behavioral consequences (Anand et al., 1999). Rat pups are born neurologically immature, and researchers agree that the first week of their lives corresponds to weeks 24–36 of gestation in human babies (Sengupta, 2011), and rat pups aged two weeks correspond to mature newborn human babies (Sengupta, 2013); thus, we decided to study the effects of painful stimuli during the first 14 days. Tactile (control for touching pup paws) and painful treatments were performed by touching pup paws with a cotton-tipped swab or by inserting a 25-gauge needle rapidly through the paw. The animal groups were designated as follows: pain (n = 22), tactile/control (n = 22), sucrose before pain (n = 24), and sucrose before tactile (n = 22). Sucrose was dissolved in distilled water at a final concentration of 25 % weight per volume (w/v). For each treatment, male and female rat pups were tested, and thus the groups were multiplied by two with a total of eight groups. Pup weights were monitored daily for the first 14 days, twice weekly until 30 days, and weekly subsequently. Weaning ended on the 28th day. Post-weaning pups were housed according to sex, with a maximum of four rats per cage. A schematic illustration of the study design is shown in Fig. 1.

2.2. Behavioral tests

Behavioral tests were performed from 4 days post-weaning, with intervals of at least 4 days between the different tests, apart from the first hot plate experiment, which was conducted 2 weeks earlier than the remaining tests. All tests were performed under standard stress-free conditions. The tests were performed by the same researcher starting with the least invasive test and ending with the most invasive test. All devices and objects used in behavioral tests were cleaned with 10 % ethanol to remove odor cues from previous subjects.

2.3. Pain sensitivity measurement

Pain sensitivity was assessed using an Orchid Hot and Cold Plate Analgesia Meter (model HC-01; Maharashtra, India). Pain sensitivity was evaluated by measuring the foot withdrawal latency (FWL). The cut-off time was 30 s and each rat underwent three trials with an interval of 20 min in between. The mean FWL (MFWL) was recorded and used for the analysis. This test was performed twice at ages of 1 month and 2 months, where the surface temperatures were set at 48 °C and 51 °C, respectively. After the pain sensitivity test, the rats were allowed one week of rest until further behavioral tests were conducted.

2.4. Rotarod

An IITC Life Science Rotarod (Model I-755; Leicester, UK) was used for rotarod testing. The experiment was performed in three trials, with 1 min rest between trials. One rat was placed on each platform with a capacity of five rats per run. The platform was set to start at a speed of 4 rpm and accelerate to 40 rpm with a cut-off time of 300 s. The device was cleaned with 10 % ethanol between runs, and the results were expressed as the average of the last two trials.

2.5. Open field

An in-house developed open field test apparatus comprising square-enclosed Plexiglas with an area of 72 cm × 72 cm and a height of 35 cm was divided into 16 identical squares (15 cm × 15 cm) using paper tape. The apparatus was wiped with 10 % ethanol before the test and each rat was then placed at the center and allowed to explore for 15 min. During this period, the movements and activities of the rats were recorded using a fixed video camera for 15 min. The following parameters were assessed manually: number of squares crossed, time spent in the central area, freezing time, grooming time, and number of rears.

2.6. Elevated plus maze (EPM)

The test was performed using an in-house developed EPM. The

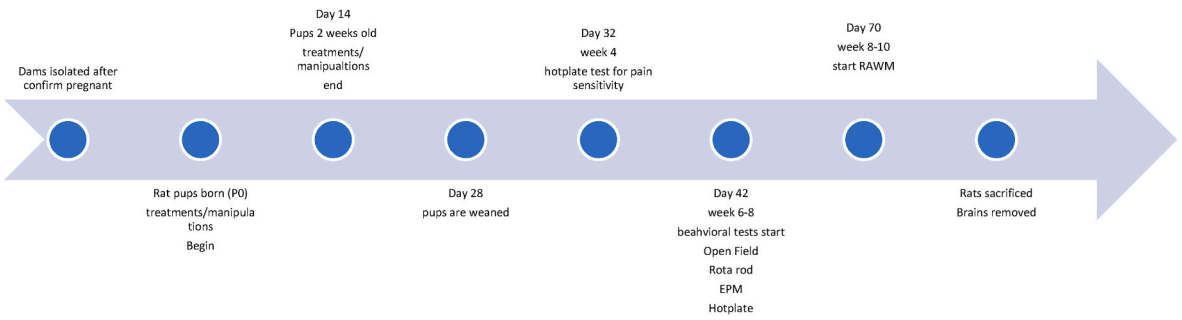

Fig. 1. Experimental protocol.

platform was 50 cm high with two closed arms (50 cm × 10 cm × 40 cm), two open arms (50 cm × 10 cm), and a central area (10 cm × 10 cm). Each rat was placed in the central area with its head directed toward the open arm. Rats were allowed to freely explore the maze for 5 min, and the entire trial was recorded using a camera. The parameters recorded comprised: number of rat entries into open arms and closed arms, and time spent in each arm.

2.7. Radial arm water maze (RAWM)

The RAWM comprised a black circular stainless-steel pool (diameter = 167 cm, height = 55 cm, depth = 43 cm) with six V-shaped stainless-steel plates (height = 49 cm, length = 55 cm) that formed a swimming field with six arms (width = 35 cm) and a platform placed at the end of a single arm, which was considered the goal. Animals were required to locate the hidden goal, where each rat had a different goal position within the arms and other rats within a single group had to locate different goals.

The test was performed in four steps: acclimation, learning, short-term memory evaluation, and long-term memory evaluation. During acclimation, six trials were conducted on the day before the memory test, and the goal was set at 2 cm higher than the water level under normal light and with no visual cues. The following day, learning was evaluated in a 12-trial test, with rest for 5 min after the sixth trial. The goal was 2 cm below the water level, with dim light and no visual cues.

The platform was placed 2 cm underwater, and each rat underwent 12 trials, which were performed six times and separated by rest for 5 min. In each trial, rats were allowed to swim freely for 1 min to reach the goal. If 1 min passed and the rat failed to find the goal, it was guided toward the goal. The number of errors was the number of entries into an arm other than the arm containing the goal. Short-term memory was evaluated as a single trial after 30 min, and long-term memory was assessed as single trials at 5 h and 24 h after the learning trials.

2.8. Statistical analysis

All statistical tests were performed using GraphPad Prism 9 (version 9 for Windows; La Jolla, CA, USA). Three-way or two-way analysis of variance (ANOVA) were conducted to detect significant differences in the data, as appropriate. The experiment examined three factors (manipulations: pain vs. tactile; treatment: sucrose vs. none; and sex: male vs. female). Interactions among the three effects were examined using three-way ANOVA, and pairwise comparisons were performed between combinations when statistically significant. If no interactions were significant, overall comparisons were performed for the three factors. Three-way ANOVA for all tests found no significant differences between males and females. Thus, the data for male and female rats were pooled, and two-way ANOVA was conducted. Post-hoc tests were performed as appropriate for each ANOVA test. All values were expressed as the mean ± standard error of the mean (SEM). $P < 0.05$ was considered to indicate a significant difference. The graphs generated by the program were used to show the results obtained by three-way ANOVA and two-way ANOVA.

3. Results

3.1. Thermal pain sensitivity on hotplate

3.1.1. Painful stimulation during infancy increased pain sensitivity to thermal stimuli in 4-week-old rats. Sucrose did not prevent painful stimulation-induced pain hypersensitivity. Furthermore, sucrose lowered the tactile pain threshold

Three-way ANOVA based on the three factors comprising manipulation (pain vs. tactile), treatment (sucrose vs. none), and sex (males vs. females) detected no significant differences between males and females, but differences were found between the manipulations and treatments (Fig. 2A).

Two-way ANOVA was conducted after pooling data for males and females, and significant differences were found between tactile and painful (manipulations) stimulated rats, as well as between (treatments) control (no pretreatment) and sucrose pretreated rats (Fig. 2B).

3.1.2. Painful stimulation during infancy increased pain sensitivity to thermal stimuli in 8-week-old rats. Sucrose did not prevent painful stimulation-induced pain hypersensitivity. Furthermore, sucrose lowered the tactile pain threshold

Three-way ANOVA based on the three factors comprising manipulation (pain vs. tactile), treatment (sucrose vs. none), and sex (males vs. females) found significant differences between pain vs. tactile, but not between treatments or sex of rats (Fig. 3A).

Two-way ANOVA using the pooled male and female data found significant differences between tactile and noxious stimulated rats, as well as between control (no pretreatment) and sucrose pretreated rats, and their interactions (Fig. 3B).

3.2. Coordination skills in rotarod test

Painful stimulation during infancy and sucrose treatment did not alter coordination skills of rats in rotarod test.

The rotarod test was used to examine motility and coordination (Ranger et al., 2019a,b; Jakkamsetti et al., 2021; Cannizzaro et al., 2022), where a rat was placed on the apparatus and the time was recorded when the rat fell off the rotarod. The test was repeated three times and the average of the second and third trials was used for the analysis. The first trial was considered a training trial, and thus it was not included in the analysis.

Three-way ANOVA found no significant differences between manipulations, or sex of rats (Fig. 4A). Two-way ANOVA based on the pooled data also found no significant differences between any of the groups, treatments, or manipulations (Fig. 4B).

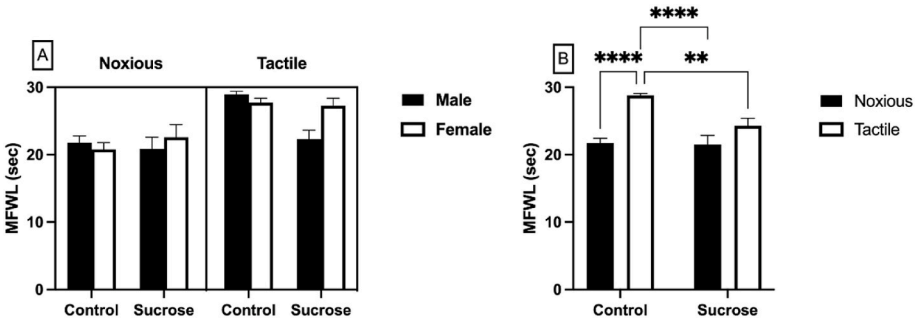

Fig. 2. Pain threshold measured as the mean foot withdrawal latency (MFWL) in seconds at 4 weeks of age. **A**. Three-way ANOVA followed by Tukey's multiple comparisons test found significant differences between painful and tactile stimuli ($F(1, 84) = 29.53$, $P < 0.0001$) but not between treatments ($F(1, 84) = 2.816$, $P = 0.0971$) or males and females ($F(1, 84) = 1.422$, $P = 0.2364$). The interactions between treatment × pain versus tactile ($F(1, 84) = 4.605$, $P = 0.0348$) and treatment × male versus female ($F(1, 84) = 5.724$, $P = 0.0190$) were also significant (mean ± SEM, N = 9–13). **B**. Two-way ANOVA followed by Sidak's multiple comparisons test for pooled male and female data found significant differences between tactile and painful (manipulations) stimulated rats ($F(1, 76) = 26.51$, $P < 0.0001$), as well as between (treatments) control (no pretreatment) and sucrose pretreated rats ($F(1, 76) = 6.140$, $P = 0.0154$), and their interactions ($F(1, 76) = 4.959$, $P = 0.0289$) (mean ± SEM, N = 15).

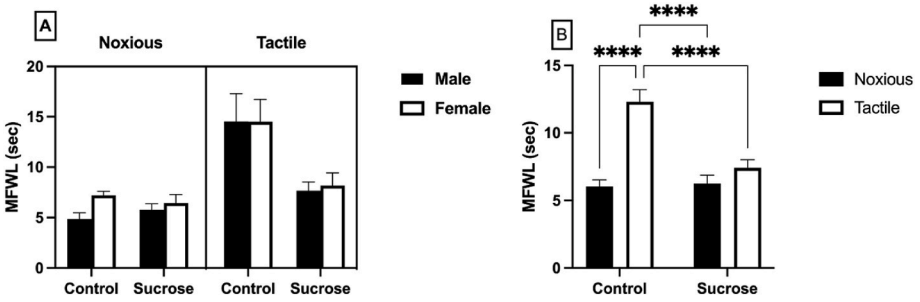

Fig. 3. Pain threshold measured as the mean foot withdrawal latency (MFWL) in seconds at 8 weeks of age. **A.** Three-way ANOVA followed by Tukey's multiple comparisons test found significant differences between painful and tactile stimuli ($F(1, 84) = 29.53$, $P < 0.0001$) but not between treatments ($F(1, 84) = 2.816$, $P = 0.0971$) or males and females ($F(1, 84) = 1.422$, $P = 0.2364$). The interactions between treatment × pain versus tactile ($F(1, 84) = 4.605$, $P = 0.0348$) and treatment × male versus female ($F(1, 84) = 5.724$, $P = 0.0190$) were also significant (mean ± SEM, N = 9–13). **B**. Two-way ANOVA followed by Sidak's multiple comparisons test using pooled male and female data found significant differences between tactile and painful (manipulations) stimulated rats ($F(1, 76) = 26.51$, $P < 0.0001$), as well as between (treatments) control (no pretreatment) and sucrose pretreated rats ($F(1, 76) = 6.140$, $P = 0.0154$), and their interactions ($F(1, 76) = 4.959$, $P = 0.0289$) (mean ± SEM, N = 15).

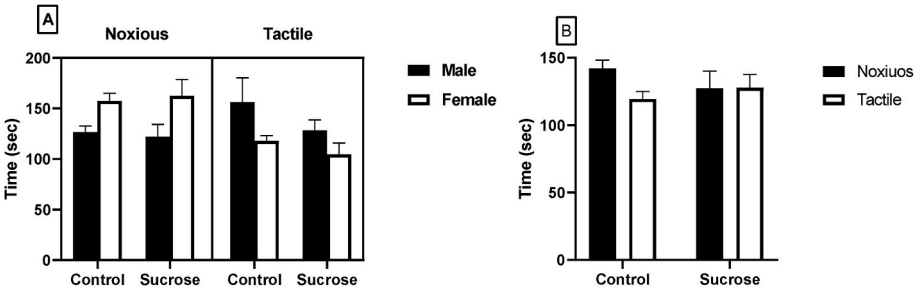

Fig. 4. Time in seconds on the rotarod (mean ± SEM, N = 14–15). **A**. Three-way ANOVA found no significant differences between manipulations ($F(1, 69) = 0.9725$, $P = 0.3275$), treatments ($F(1, 69) = 2.200$, $P = 0.1426$), or sex of rats ($F(1, 69) = 0.05508$, $P = 0.8151$). **B**. Two-way ANOVA based on the pooled data also found no significant differences between any of the groups, treatments ($F(1, 52) = 0.1094$, $P = 0.7422$), or manipulations ($F(1, 52) = 1.360$, $P = 0.2489$).

3.3. Locomotion and anxiety in the open field

Painfully stimulated rats exhibited mixed results in the open field test, where the number of rears and number of squares crossed were higher, but grooming and time in the center were unchanged.

The open field test monitors "anxiety-related behaviors, exploratory behavior, and emotionality" in rats (Sestakova et al., 2013). The recorded behaviors comprised the number of rears, number of squares crossed, total time spent in the center area, and grooming time. Three-way ANOVA was used to determine factors with significant effects, before conducting two-way ANOVA with appropriate post-hoc tests. The three-way ANOVA results and figures are shown in the Supplementary Material. The two-way ANOVA results were recorded.

3.3.1. Number of rears

Painfully stimulated rats had a higher number of rears compared with tactile stimulated rats. Sucrose appeared to normalize the effect of pain stimulation on tactile values.

Three-way ANOVA found significant differences between treatments and manipulations, but not between sexes. Significant interactions were found between treatment × (noxious vs. tactile) and treatment × (male vs. female). Further details are shown in Fig. 1SA.

Two-way ANOVA based on the pooled data also found significant differences between treatments, manipulations, and their interactions (Fig. 5A).

3.3.2. Squares crossed

Painfully stimulated rats crossed the highest number of squares, but the number was not significantly different from that by tactile-stimulated rats. Sucrose treatment decreased the number of squares crossed by both painfully and tactile stimulated rats.

Three-way ANOVA found significant differences between treatments and manipulations (noxious vs. tactile), but not between sexes (male vs. female). Significant interactions were found between (pain vs. tactile) × (male vs. female). Further details are shown in Fig. 1SB.

Two-way ANOVA based on the pooled data found significant differences between treatments, and sucrose with and without pain stimulation reduced the number of squares crossed (Fig. 5B).

3.3.3. Time in center

Painful stimulation and sucrose pretreatment did not affect the time spent in the center. Sucrose in tactile stimulated rats increased the time in the center compared with non-treated tactile-stimulated rats.

Three-way ANOVA showed that the only significant interaction was treatment × (pain vs. tactile). Further details are provided in Fig. 1SC.

Two-way ANOVA based on the pooled data also showed that only the interaction between treatment and manipulation was significant ($F(1, 55) = 4.593$, $P = 0.0365$) (Fig. 5C).

3.3.4. Grooming time

Painful stimulation and sucrose pretreatment did not affect the time spent grooming.

Three-way ANOVA found no significant differences between treatment, manipulation, or sex (Fig. 1SD).

Two-way ANOVA based on the pooled data also found no significant differences (Fig. 5D).

3.4. EPM

The EPM was employed to examine anxiety-like behaviors. The EPM test allowed us to detect anxiety-related activities that might not have been observed in other tests, such as the open field test. The EPM detects elements of both passive and active evasion at the same time. The variables recorded in the EPM were the number of entries into each arm, time spent in each arm, and time spent in the central square.

3.4.1. Painfully stimulated rats spent most of their time in the open arms. They also entered the open arms more frequently than sucrose-treated rats

Three-way ANOVA based on the number of entries into the open arms detected significant differences between treatments and manipulations, but not between males and females, as well as the interactions between treatment × N vs. T × male vs. female. Further details are shown in Fig. 2SA.

Two-way ANOVA based on the pooled data (males plus females) found significant differences between the treatments and manipulations (Fig. 6A), where pain-stimulated rats without sucrose pretreatment entered the open arms most frequently.

Three-way ANOVA based on the time spent in open arms found significant differences between treatments, manipulations, and the interaction between treatment × manipulation × male vs. female, but not between males and females. Further details are shown in Fig. 2SB.

Two-way ANOVA detected significant differences between treatments and manipulations (Fig. 6B), where pain-stimulated rats without sucrose pretreatment spent more time in the open arms.

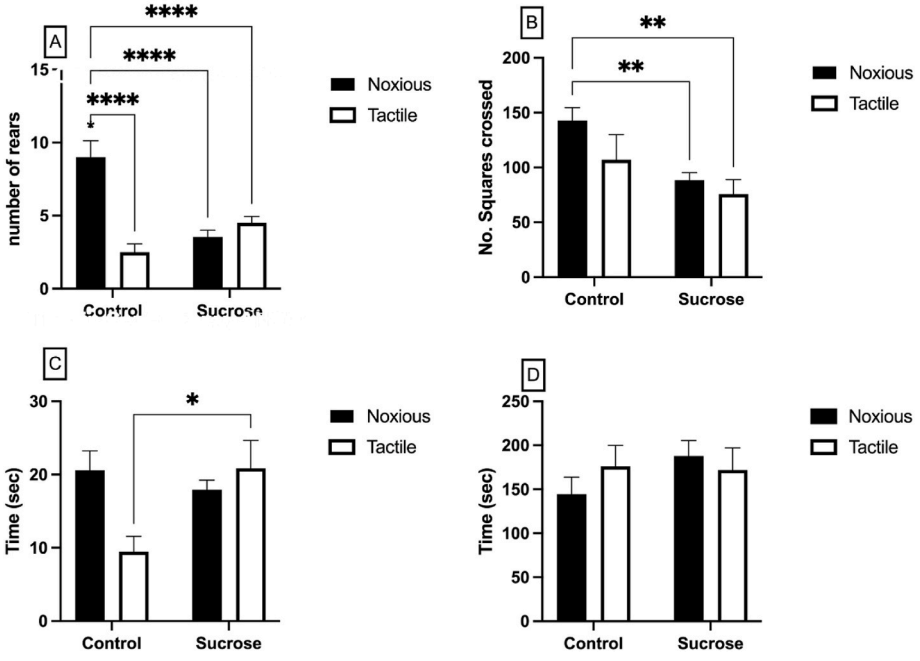

Fig. 5. Open field test results. A. Number of rears: painfully stimulated rats had a significantly higher number of rears (data: mean ± SEM, N = 12–20). Two-way ANOVA based on the pooled data found significant differences between treatments ($F(1, 62) = 6.785$, $P = 0.0115$), manipulations ($F(1, 62) = 17.56$, $P < 0.0001$), and their interactions ($F(1, 62) = 31.64$, $P < 0.0001$). **B.** Number of squares crossed: painfully stimulated, untreated rats crossed a higher number of squares (data: mean ± SEM, N = 7–20). Two-way ANOVA based on the pooled data found significant differences between treatments ($F(1, 57) = 10.17$, $P = 0.0023$). **C.** Time spent in the center of the open field in seconds (data: mean ± SEM, N: 11–19). Two-way ANOVA based on the pooled data showed that only the interaction between treatment and manipulation was significant ($F(1, 55) = 4.593$, $P = 0.0365$). **D.** Grooming time in seconds (data: mean ± SEM, N = 11–15). Two-way ANOVA based on the pooled data found no significant differences between treatments ($F(1, 48) = 0.8364$, $P = 0.3650$), manipulations ($F(1, 48) = 0.1342$, $P = 0.7157$), and their interaction ($F(1, 48) = 1.235$, $P = 0.2720$).

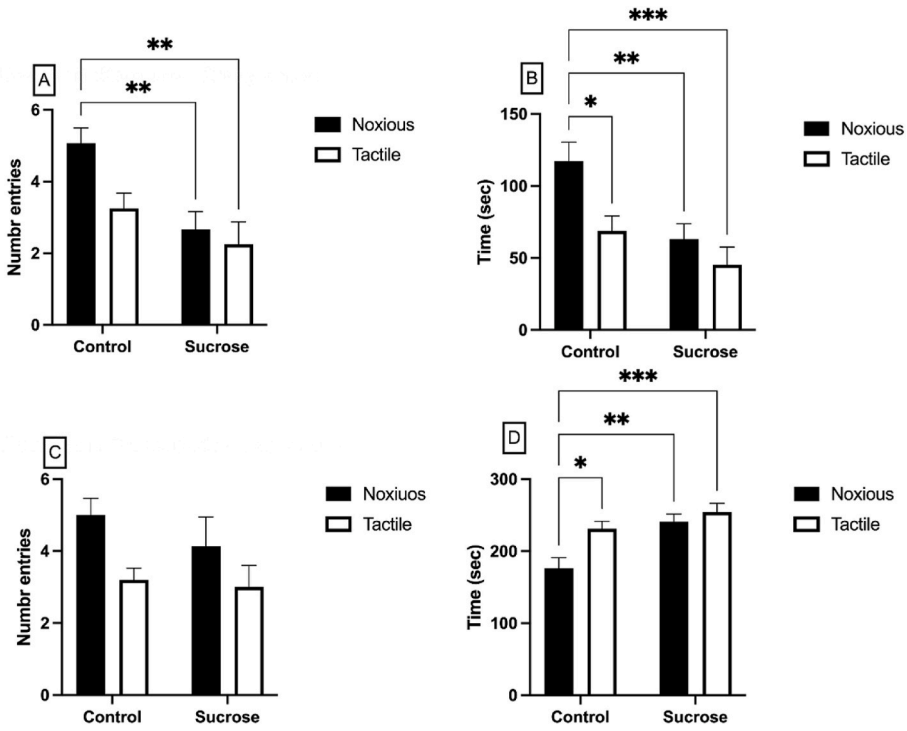

Fig. 6. Elevated plus maze test results. **A.** Number of entries into open arms: painfully stimulated rats spent more time in the open arms than sucrose-treated tactile, and pain stimulated rats. Data represent mean ± SEM (N = 12–15). Two-way ANOVA found significant differences between the treatments (F (1, 49) = 11.59, P = 0.0013) and manipulations (F (1, 49) = 5.010, P = 0.0298). **B.** Time spent in open arms; painfully stimulated rats spent more time in the open arms than tactile-stimulated, and sucrose-treated tactile and pain stimulated rats. Data represent mean ± SEM (N = 12–15). Two-way ANOVA found significant differences between treatments (F (1, 49) = 10.77, P = 0.0019) and manipulations (F (1, 49) = 7.849, P = 0.0073). **C.** Number of entries into closed arms: no significant differences were found between pain or tactile-stimulated rats or sucrose-treated rats. Data represent mean ± SEM (N = 12–15). Two-way ANOVA found no significant difference between treatments (F (1, 47) = 0.7010, P = 0.4067) but a significant difference between manipulations (F (1, 47) = 5.301, P = 0.0258). **D.**Mean time spent in closed arms: painfully stimulated rats spent less time in the closed arms than tactile stimulated and sucrose-treated tactile, and pain stimulated rats. Data represent mean ± SEM (N: 12–15). Two-way ANOVA found significant differences between treatments (F (1, 49) = 12.52, P = 0.0009) and manipulations (F (1, 49) = 7.607, P = 0.0082).

3.4.2. Painfully stimulated rats spent the shortest time in the closed arms. They entered the closed arms more frequently, but there were no significant differences between groups

Three-way ANOVA based on the number of entries into closed arms found no significant differences between treatments, manipulations, or between males and females. Further details are provided in Fig. 2SC.

Two-way ANOVA detected no significant difference between treatments but a significant difference between manipulations, although a pairwise comparison test found no significant difference (Fig. 6C). Thus, sucrose pretreatment did not result in any significant differences.

Three-way ANOVA based on the time spent in the closed arms found significant differences between treatments, manipulations, and the interaction between treatment × manipulation × male vs. female, but not between males and females. Further details are provided in Fig. 2SD.

Two-way ANOVA found significant differences between treatments and manipulations (Fig. 6D)**.** Pain resulted in rats spending less time in the closed arms of the maze compared with tactile and sucrose treatment.

3.5. Spatial learning and memory in water maze tests

Sucrose pretreatment with or without pain stimulation increased the number of errors in short- and long-term memory tests.

Short-term memory tests were conducted for rats 30 min after completing the training session in the RAWM. Rats with painful stimulation during infancy and tactile stimulated rats did not differ significantly in terms of the number of errors, but sucrose increased the number of errors in both tactile and pain stimulated rats compared with tactile-only rats. Thus, sucrose had negative effects on short- and long-term memory in rats regardless of painful or tactile stimulation.

Three-way ANOVA found significant differences between treatments and the interaction between treatment × pain vs. tactile, but not between pain vs. tactile and male versus female. Further details are provided in Fig. 3SA. Two-way ANOVA based on the pooled data only found a significant difference between treatments (Fig. 7A).

Long-term memory tests were conducted 5 h after the training session for the first long-term memory tests. The results were similar to those for the short-term memory tests at 30 min.

Three-way ANOVA found a significant difference between treatments but not between pain vs. tactile or male vs. female (Fig. 3SB). Two-way ANOVA found a significant difference between treatments (F (1, 62) = 13.00, P = 0.0006) (Fig. 7B).

Long-term memory tests conducted 24 h later obtained similar results to those at 5 h and 30 min after training.

Three-way ANOVA found a significant difference between treatments but not for pain versus tactile or male and female (Fig. 3SC). Two-way ANOVA found a significant difference between treatments (F (1, 56) = 11.58, P = 0.0012) for pain versus tactile (Fig. 7C).

4. Discussion

This study tested whether sucrose administration could prevent the adverse effects of neonatal painful stimulation in Sprague–Dawley rats, and the long-term effects of painful stimulation and sucrose pretreatment were assessed in several behavioral tests. The results showed that painful stimulation during infancy had none or some negative effects on behavioral performance by rats later in life. Neonatal painful stimulation increased the sensitivity to thermal stimulation at four and eight

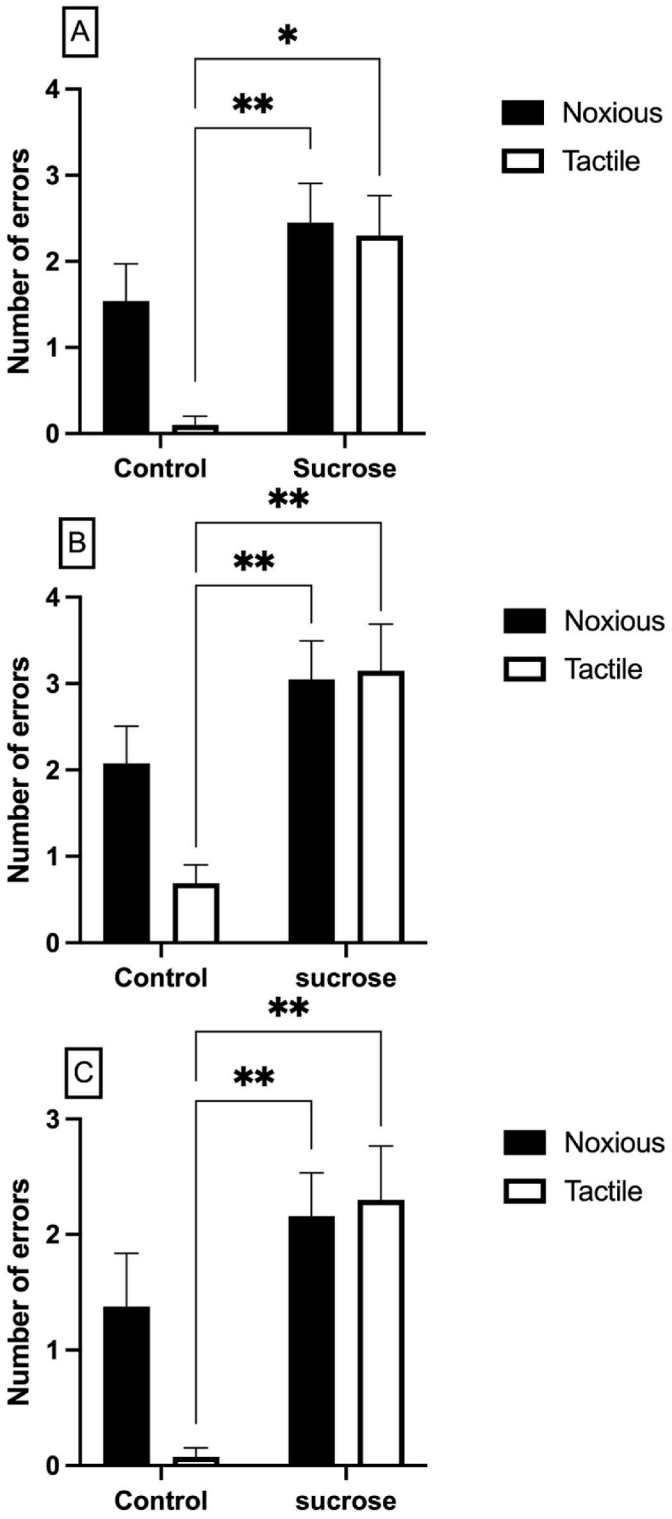

Fig. 7. Rat performance in radial arm water maze (mean ± SEM, N = 9–20). A. Short-term memory (30 min). Two-way ANOVA based on the pooled data found a significant difference between treatments ($F(1, 59) = 10.78$, $P = 0.0017$). B. Long-term (5 h) memory. Two-way ANOVA found a significant difference between treatments ($F(1, 62) = 13.00$, $P = 0.0006$). Tactile-stimulated, untreated rats had the lowest number of errors, which differed significantly from those by sucrose-treated rats. C. Long-term (24 h) memory. Two-way ANOVA found a significant difference between treatments ($F(1, 56) = 11.58$, $P = 0.0012$) for pain versus tactile.

weeks of age. In open field tests, the number of rears and number of squares crossed were increased by painful stimulation. In the EPM, painfully stimulated rat pups entered more open arms and spent more time in open arms. Neonatal pain did not affect the spatial memory of rats according to water maze tests. However, sucrose pretreatment with or without pain had some interesting effects.

Sucrose pretreatment did not alleviate pain induced sensitivity to thermal stimulation, or improve spatial learning and memory, but instead sucrose with or without pain made learning and memory worse.

The results obtained in this study agree with our previous demonstration that painful stimulation in this rat model can lower the threshold for thermal stimulation later in life (Nuseir et al., 2015, 2017, 2021). In particular, painfully stimulated rats exhibited higher sensitivity to thermal stimulation at both 4 weeks of age (early adolescence) and 8 weeks of age (adulthood). However, in contrast to our hypothesis, sucrose pretreatment did not prevent this increase in thermal sensitivity, and it even lowered the threshold for tactile-stimulated rats. By contrast, our previous studies suggested that sucrose could be beneficial for reversing the effects of painful stimulation (Nuseir et al., 2015, 2017, 2019, 2021).

In the rotarod test, which measures coordination, we found no significant differences between pain and tactile stimulated rats, or between sucrose-treated and untreated rats. Therefore, painful stimulation or sucrose pretreatment did not affect the coordination skills of rats later in life. By contrast, a study of human preterm infants found that glucose pretreatment negatively affected motor functions at 18 months of age (Schneider et al., 2018).

The open-field test was used to examine anxiety-related behaviors, curious behaviors, and emotionality in rats. Several parameters were assessed in the open field test and the results showed that rats with painful stimulation during infancy had the highest number of rears, thereby indicating anxiety-like or probing and curious behavior. However, sucrose pretreatment effectively neutralized this behavior. Rearing has been used widely as a marker of anxiety or exploratory behavior in rodents (Sestakova et al., 2013; Seibenhener and Wooten, 2015; Rudolfová et al., 2022). Rats that were painfully stimulated during infancy appeared to exhibit some anxiety-like behavior during early adulthood. The number of squares crossed by rats was highest for painfully stimulated rats, but they also appeared to spend more time in the center. However, sucrose appeared to lower the number of squares crossed even in tactile stimulated rats. In addition, sucrose treatment increased the time in the center for both pain and tactile stimulated rats. Sucrose pretreatment before tactile stimulation had a significantly different effect compared with tactile only stimulation in rats. These results suggest that anxiety-like behaviors can manifest differently in the same test. Pain during infancy can lead to increased anxiety in both humans and animals, and treating this pain can mitigate anxiety (Walker, 2017; Steinbauer et al., 2022).

The EPM test allowed us to detect anxiety-related behaviors that might not have been detected by other tests because it tested for elements of both passive and active avoidance at the same time. Less entries and time spent in the open arms of the maze indicated lower anxiety, but painfully stimulated rats entered and spent more time in the open arms, which was reversed by sucrose administration. In addition, the painfully stimulated rats spent the lowest amount of time in the closed arms, which was normalized by sucrose pretreatment. It is possible that sucrose induced anxiety-like behaviors that manifested later in life as spending more time in closed arms and entering open arms less frequently. This is an important finding and previous studies of both humans and animals also showed that sweet pretreatment in preterm infants resulted in poorer neurodevelopmental outcomes later in life (Schneider et al., 2018; Ranger et al., 2019a,b).

The different results obtained in the open field and EPM tests might be explained by the types of anxiety assessed by each test. In the open field test, the anxiety exhibited by exploring rats is probably inherent anxiety, whereas the EPM test measures situational anxiety (de Kort

et al., 2021). In addition, the Sprague–Dawley rat strain used in the present study has an anxiolytic profile (Rex et al., 2004). Furthermore, previous research suggests a different role for pain during infancy because it reduces responses to stressful and anxiogenic stimuli later in life via interference in the hypothalamic–pituitary–adrenal axis (Victoria and Murphy, 2016).

The results obtained in the water maze test employed for assessing short- and long-term spatial memory and cognition showed that tactile stimulated and untreated rats had the lowest number of errors in the water maze test compared with painfully stimulated rats (but the difference was not statistically significant). However, sucrose pretreatment augmented memory deficiency in both tactile and pain stimulated rats. By contrast, our previous study showed that painful stimulation caused long-term memory deficiency, and sucrose pretreatment could prevent or reverse this detrimental effect (Nuseir et al., 2015, 2017). Ranger et al. (2019a,b) demonstrated that mice treated with sucrose before painful stimulation during infancy had worse outcomes in anxiety and cognitive tests in adulthood (Ranger et al., 2019a,b). Sucrose also resulted in poorer outcomes in tactile or handling-only mice. However, the same results were observed in human infants, where glucose did not relieve the effects of early pain on brain development (Schneider et al., 2018).

It should be noted that several differences between our previous research and the current study may explain this discrepancy. In particular, we used Sprague–Dawley rats instead of Wistar rats, and sucrose was administered to tactile stimulated rats and not only pain stimulated rats in the experimental protocol.

Differences in rat strains that are probably related to genetic variables have been observed and examined in several research models (Becker et al., 2016). For example, Sprague–Dawley rats were shown to be more susceptible to neurotoxicity and memory deficits than Wistar Han rats (Zmarowski et al., 2012). Differences in exploratory and emotionality behaviors were found between two strains of rats in anxiety tests (Ramos et al., 2002). Other studies also demonstrated the importance of the rat strain for behavioral profiles and behaviors in several tests (Ramos et al., 1997; López-Rubalcava and Lucki, 2000; van der Staay et al., 2009). Furthermore, differences in developmental–behavioral characters were observed between Wistar and Sprague–Dawley rats, which are the two strains used in our facility, and they are commonly applied in pharmacological and behavioral studies (Asano, 1986).

It is well established that preterm and term infants differ in terms of their response to repetitive painful stimuli such as heel lance. Moreover, term infants exhibit habituation to painful stimuli, whereas preterm infants do not habituate to these repeated painful stimuli (Rupawala et al., 2023). In the present study, rat pups were exposed to painful stimuli for the first 14 days, which corresponded to both periods (preterm and term).

Overall, our results suggested that sucrose administration during infancy had complex effects on anxiety-related behaviors and cognitive function in rats. Sucrose did not prevent the increased thermal sensitivity observed in rats that had been painfully stimulated, but it did appear to reverse some anxiety-related behaviors in the open field and EPM tests. Another important result was that sucrose was ineffective in mitigating the negative effects of painful stimulation, but some outcomes of sucrose treatment were also adverse. Sucrose and other sugars given to preterm infants seem to reduce the physical response to acute pain but not the long-term effects of this pain according to human and animal studies (Schneider et al., 2018; Ranger et al., 2019a,b; Ramírez-Contreras et al., 2021).

These reports showing the negative impacts of sucrose and other sweet-tasting solutions given during infancy, particularly in preterm infants, on lessening procedural pain in the neonatal intensive care unit (Mencía et al., 2022; Fulkoski et al., 2023) should be given greater consideration, and more studies are required to explore this negative effect. In addition, more research is needed to explore the mechanisms associated with the long-term harmful effects of pain, as well as the efficacy and safety of using sucrose in neonatal intensive care units.

Limitations and further directions

Water treated rat pups separated into two age groups corresponding to preterm human infants (P0–P7) and term human infants (P8–P15) will be investigated in our next project.

Ethical statement

All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize suffering and stress, such as returning pups to their cages between manipulations and using a minimum number of rats per experiment. All experimental procedures were approved by the Animal Care and Use Committee (ACUC) of the Jordan University of Science and Technology (Grant Number: 2022/53).

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Availability of data and material

Raw data are available upon request.

Authors contributions

KN, KA, AA: Conceptualization and methodology. NA, RO: Investigation. KN, KA, AA: Formal analysis. KN, KA, MK: Validation. KN, KO: Writing - Original Draft. KN, AA: Writing - Review & Editing.

Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crphar.2024.100176.

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